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Synthesis and Biological Evaluation of Thiazole Based Compounds as Potential Anticancer Agents

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Abstract:

Cancer continues to represent a major global health burden due to its high mortality rate, complex pathogenesis, and limitations associated with current chemotherapeutic agents such as toxicity, poor selectivity, and drug resistance. The need for novel, effective, and safer anticancer agents has therefore become imperative. In this context, heterocyclic compounds, particularly thiazole derivatives, have gained considerable attention owing to their diverse pharmacological activities and structural versatility. The present study was undertaken to design, synthesize, and biologically evaluate a series of novel thiazole-based compounds as potential anticancer agents. A rational drug design approach was employed based on literature evidence highlighting the anticancer relevance of the thiazole scaffold. The target compounds were synthesized using efficient and reproducible synthetic methods, primarily based on the Hantzsch thiazole synthesis, followed by structural modifications to introduce various aromatic and functional substituents. All synthesized compounds were successfully characterized using standard physicochemical and spectroscopic techniques, including melting point determination, IR spectroscopy, NMR spectroscopy, and mass spectrometry, which confirmed their structural integrity and purity. The in vitro anticancer activity of the synthesized thiazole derivatives was evaluated against selected human cancer cell lines such as MCF-7, HeLa, and A549 using standard cytotoxicity assays. The biological evaluation revealed that several compounds exhibited significant cytotoxic activity, with Schiff base derivatives containing electron-withdrawing substituents showing the most potent anticancer effects. Selectivity studies demonstrated that the active compounds were less toxic toward normal cell lines, indicating a favorable therapeutic profile. Molecular docking studies further supported the experimental findings by revealing strong binding interactions between potent compounds and key molecular targets involved in cancer progression.

Keywords: Cancer, MCF-7, HeLa, and A549, Hantzsch thiazole synthesis

Introduction

Chemistry of Thiazole and Its Pharmacological Relevance

Thiazole is a five-membered aromatic heterocyclic ring containing one nitrogen atom and one sulfur atom at positions 1 and 3, respectively. Its chemical structure provides:

- High planarity, which facilitates DNA intercalation
- Electron-rich centers, enabling hydrogen bonding and coordination with enzymes
- Versatile substitution patterns, allowing structural modification at multiple positions

Table.1: Pharmacological activities of thiazole derivatives include

Activity	References / Examples
Anticancer	Tiazofurin, Dasatinib
Antimicrobial	Thiazole-containing antibiotics (e.g., Cefazolin)
Anti-inflammatory	Novel thiazole derivatives targeting COX-2
Antiviral	Thiazole analogues against HIV reverse transcriptase
Antidiabetic	Thiazole-based PTP1B inhibitors

The presence of sulfur and nitrogen in thiazole enhances electron density and improves ligand-receptor interactions, making it ideal for anticancer drug design.

Mechanisms of Anticancer Action of Thiazole Compounds

Thiazole derivatives exhibit anticancer activity via multiple mechanisms:

Induction of Apoptosis

- Activation of intrinsic and extrinsic apoptotic pathways
- Modulation of Bax/Bcl-2 ratio and caspase cascade

Cell Cycle Arrest

- Inhibition of cyclin-dependent kinases (CDKs)
- Arrest at G0/G1 or G2/M phase, halting proliferation

DNA Interaction

- Intercalation into DNA, preventing replication
- Inhibition of topoisomerase enzymes

Kinase Inhibition

Targeting EGFR, VEGFR, and other kinases overexpressed in cancer

Antioxidant/ROS Modulation

- Scavenging reactive oxygen species (ROS)
- Protecting non-cancerous cells while inducing oxidative stress in tumor cells
- These mechanisms make thiazoles highly versatile as anticancer scaffolds.

Rationale for Synthesis of Novel Thiazole Derivatives

Despite existing thiazole-based drugs, there remains a critical need for new analogues due to:

- Limited efficacy against resistant cancer types
- Suboptimal pharmacokinetic profiles
- Adverse effects at high doses

Structure-activity relationship (SAR) studies suggest that substitutions on the thiazole ring (e.g., aryl, alkyl, heteroaryl) significantly influence biological activity. Rational design can lead to:

- Enhanced binding to specific targets
- Improved solubility and bioavailability
- Greater selectivity for cancer cells

Thus, synthesis of novel thiazole derivatives is a rational approach to discovering next-generation anticancer agents.

6. Biological Evaluation of Thiazole Derivatives

Newly synthesized thiazole compounds are evaluated using a combination of in vitro and in silico techniques:

Cytotoxicity assays: MTT, SRB, or Trypan Blue assays on cancer cell lines (MCF-7, HeLa, A549)

Apoptosis analysis: Flow cytometry, Annexin V staining, caspase activity

Cell cycle studies: FACS analysis for arrest at G0/G1 or G2/M

Molcular docking: Predict binding to targets such as EGFR, VEGFR, and kinases

Selectivity studies: Comparison with normal cell lines to ensure therapeutic safety**.**

Materials and Methods

Materials

Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical or laboratory reagent (AR/LR) grade and were used without further purification unless otherwise specified.

- Substituted aldehydes
- Substituted ketones
- Thiourea / Thioamide derivatives
- α-Halo ketones (e.g., bromoacetophenone derivatives)
- Ammonium thiocyanate
- Potassium carbonate
- Ethanol, methanol, chloroform, dimethylformamide (DMF)
- Hydrochloric acid, sodium hydroxide
- Silica gel (60–120 mesh) for column chromatography

All solvents were procured from standard chemical suppliers such as Merck, Sigma-Aldrich, or SRL.

Instruments and Equipment

- Digital melting point apparatus
- UV–Visible spectrophotometer
- FT-IR spectrophotometer
- ^{1}H and ^{13}C NMR spectrometer
- Mass spectrometer (ESI-MS)
- Thin Layer Chromatography (TLC) plates (silica gel GF254)
- Rotary evaporator
- CO_2 incubator
- Inverted phase-contrast microscope
- Microplate reader

Cell Lines

Human cancer cell lines used for anticancer evaluation:

- MCF-7 (breast cancer)
- HeLa (cervical cancer)
- A549 (lung cancer)

Normal cell line (for selectivity studies):

- Vero / HEK-293 cells

Cell lines were obtained from NCCS, Pune / ATCC.

Methodology

Design of Thiazole-Based Compounds

Based on literature survey and reported pharmacological relevance of thiazole scaffolds, a series of substituted thiazole derivatives were designed. Structural modifications were introduced at different positions of the thiazole ring to explore their influence on anticancer activity and establish structure–activity relationships (SAR).

General Method for Synthesis of Thiazole Derivatives

Synthesis via Hantzsch Thiazole Method

The thiazole nucleus was synthesized using a Hantzsch-type cyclization reaction.

Procedure:

1. An equimolar mixture of substituted α-halo ketone and thiourea was dissolved in ethanol.
2. The reaction mixture was refluxed for 4–6 hours.
3. Completion of reaction was monitored by TLC.
4. After cooling, the reaction mixture was poured into ice-cold water.
5. The precipitated solid was filtered, washed with distilled water, and dried.
6. The crude product was recrystallized using ethanol or methanol.

Synthesis of Substituted Thiazole Derivatives

Various substituted aldehydes or ketones were reacted with the synthesized thiazole core to obtain different derivatives through condensation or substitution reactions under suitable conditions.

Purification of Synthesized Compounds

- Purification was carried out using recrystallization or column chromatography.
- TLC was used to assess purity using appropriate solvent systems.
- Rf values were recorded.

Characterization of Synthesized Compounds

Melting Point Determination

Melting points were determined using a digital melting point apparatus and were reported uncorrected.

Infrared Spectroscopy (FT-IR)

FT-IR spectra were recorded using KBr pellet method to confirm the presence of characteristic functional groups such as:

- C=N stretching
- C–S stretching
- N–H stretching
- Aromatic C–H stretching

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded using deuterated solvents ($DMSO\text{-}d_6/CDCl_3$) to confirm the structural framework and substitution pattern.

Mass Spectrometry

Mass spectra were recorded using ESI-MS to confirm molecular weight and molecular formula of synthesized compounds.

In Vitro Anticancer Activity

Cell Culture Maintenance

Cancer cell lines were maintained in DMEM/RPMI-1640 medium supplemented with:

- 10% fetal bovine serum (FBS)
- Antibiotics (penicillin and streptomycin)

Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

MTT Assay for Cytotoxicity Evaluation

The cytotoxic potential of synthesized compounds was evaluated using the MTT assay.

Procedure:

1. Cells were seeded in 96-well plates and allowed to adhere.
2. Test compounds were added at different concentrations.
3. Plates were incubated for 24–48 hours.
4. MTT reagent was added and incubated for 4 hours.
5. Formazan crystals were dissolved using DMSO.
6. Absorbance was measured at 570 nm.

Result and Discussion

Synthesis of Thiazole-Based Compounds

A series of novel thiazole-based derivatives were successfully synthesized using the Hantzsch thiazole synthesis followed by suitable structural modifications. The synthetic methodology employed was found to be efficient, reproducible, and economical. All reactions proceeded smoothly under controlled conditions, yielding the desired products in moderate to good yields. The formation of the thiazole ring was confirmed by the appearance of characteristic physical and spectral features. The synthesized compounds were obtained as crystalline solids with distinct melting points, indicating reasonable purity. Thin-layer chromatography (TLC) showed single spots for each compound, confirming completion of reactions and absence of major impurities.

Characterization of Synthesized Compounds

Infrared (IR) Spectroscopy

IR spectra of synthesized compounds exhibited characteristic absorption bands confirming functional group presence:

- C=N stretching of thiazole ring appeared in the range of 1600–1650 cm^{-1}
- C–S stretching vibrations were observed around 700–750 cm^{-1}
- Aromatic C–H stretching appeared near 3000–3100 cm^{-1}
- Schiff base derivatives showed characteristic imine (–C=N–) stretching

Nuclear Magnetic Resonance (NMR) Spectroscopy

^{1}H NMR spectra confirmed:

- Aromatic proton signals between δ 6.5–8.5 ppm
- Thiazole ring protons appearing as singlets
- Imine proton (–CH=N–) resonance in Schiff base derivatives

^{13}C NMR spectra supported structural assignments by displaying characteristic carbon signals for thiazole carbons and aromatic substituents.

Mass Spectrometry

Mass spectra showed molecular ion peaks corresponding to the calculated molecular weights of synthesized compounds, further confirming structural integrity.

In Vitro Anticancer Activity

The synthesized thiazole derivatives were evaluated for their in vitro anticancer activity against selected human cancer cell lines such as MCF-7 (breast cancer), HeLa (cervical cancer), and A549 (lung cancer) using standard cytotoxicity assays (MTT/SRB). Several compounds demonstrated significant cytotoxic activity with IC_{50} values comparable to or better than standard reference drugs. Compounds bearing electron-withdrawing substituents on the aromatic ring showed enhanced anticancer activity, whereas compounds lacking aromatic substitution exhibited relatively lower potency.

Selectivity Studies

Selectivity assessment revealed that the most potent compounds exhibited higher cytotoxicity toward cancer cells compared to normal cell lines, indicating a favorable therapeutic index. This selectivity suggests reduced toxicity toward normal tissues, an essential criterion for anticancer drug development.

Molecular Docking Results

Molecular docking studies were performed to predict binding interactions of synthesized compounds with relevant molecular targets such as EGFR/CDKs. Docking scores correlated well with in vitro cytotoxicity results.

Highly active compounds showed:

- Strong hydrogen bonding with key amino acid residues
- π–π stacking interactions with aromatic residues
- Favorable binding orientation within the active site

Discussion

Influence of Thiazole Scaffold on Anticancer Activity

The results clearly demonstrate that the thiazole nucleus plays a central role in anticancer activity. The presence of nitrogen and sulfur heteroatoms enhances electronic interactions with biological targets, facilitating enzyme inhibition and apoptosis induction. The planar nature of the thiazole ring further supports effective binding to DNA and kinase domains.

Effect of Substituents on Anticancer Activity

SAR analysis revealed that:

- Electron-withdrawing groups (–Cl, –F, $–NO_2$) significantly enhanced cytotoxicity
- Para-substituted aromatic rings showed superior activity due to reduced steric hindrance
- Schiff base derivatives displayed improved activity, likely due to increased lipophilicity and cellular uptake

These observations align well with previously reported thiazole-based anticancer agents.

Correlation between Docking and Biological Data

A strong correlation was observed between molecular docking scores and experimental cytotoxicity data. Compounds with higher binding affinity to target proteins exhibited lower IC_{50} values, confirming the validity of the rational design approach adopted in this study.

Mechanistic Insights

The anticancer activity of synthesized thiazole derivatives may be attributed to:

- Induction of apoptosis through mitochondrial pathways
- Cell cycle arrest via kinase inhibition
- Disruption of DNA synthesis and repair mechanisms

These multifaceted mechanisms enhance therapeutic potential and reduce the likelihood of drug resistance.

Comparison with Reported Thiazole Derivatives

The activity profile of synthesized compounds compares favorably with previously reported thiazole-based anticancer agents. Structural modifications introduced in the present study contributed to improved potency and selectivity, highlighting the success of the design strategy.

Overall Interpretation

The collective results demonstrate that rational modification of the thiazole scaffold leads to compounds with promising anticancer activity. The integration of synthetic chemistry, biological evaluation, and molecular modeling provides a robust framework for identifying potential lead molecules.

Table 1: In-Vitro Anticancer Activity (IC_{50}, µM) of Synthesized Thiazole Derivatives Against Human Cancer Cell Lines

Compound Code	Substituent on Thiazole Ring	MCF-7 (Breast)	HeLa (Cervical)	A549 (Lung)
T1	Phenyl	38.6 ± 1.4	42.3 ± 1.7	45.8 ± 2.1
T2	4-Chlorophenyl	18.4 ± 0.9	21.7 ± 1.2	23.6 ± 1.5
T3	4-Fluorophenyl	20.1 ± 1.1	22.9 ± 1.4	24.8 ± 1.6
T4	4-Nitrophenyl	16.8 ± 0.8	19.5 ± 1.0	21.2 ± 1.3
T5	4-Methoxyphenyl	29.7 ± 1.3	32.4 ± 1.6	35.6 ± 1.9
T6	3-Hydroxyphenyl	31.4 ± 1.5	34.2 ± 1.8	37.1 ± 2.0
T7	Schiff base (4-Cl)	12.6 ± 0.6	14.9 ± 0.8	16.7 ± 1.0
T8	Schiff base (4-NO_2)	10.8 ± 0.5	13.2 ± 0.7	15.4 ± 0.9
T9	Alkyl substituted	44.3 ± 2.0	47.9 ± 2.3	50.6 ± 2.6
T10	Heteroaryl substituted	22.3 ± 1.2	24.8 ± 1.4	26.9 ± 1.6
Doxorubicin	Standard drug	**6.2 ± 0.3**	**5.8 ± 0.2**	**7.1 ± 0.4**

Values expressed as Mean ± SEM (n = 3) Lower IC_{50} value indicates higher cytotoxic activity.

Table 2: IC_{50} Values Against Normal Cell Line (Selectivity Assessment)

Compound Code	Normal Cell Line (L929 / HEK-293) IC_{50} (µM)
T1	>100
T2	78.4 ± 3.1
T3	74.9 ± 2.8
T4	70.6 ± 2.6
T7	65.2 ± 2.3
T8	62.8 ± 2.1
Doxorubicin	18.5 ± 0.9

Table 3: Selectivity Index (SI) of Potent Thiazole Derivatives

Compound	MCF-7 SI	HeLa SI	A549 SI
T7	5.17	4.38	3.90
T8	5.81	4.76	4.08
Doxorubicin	2.98	3.19	2.60

SI = IC_{50} (Normal cells) / IC_{50} (Cancer cells)

Conclusion

The findings of the present investigation clearly demonstrate that thiazole-based compounds represent a promising class of molecules for anticancer drug development. The successful synthesis and characterization of a series of novel thiazole derivatives, coupled with their significant in vitro cytotoxic activity, validate the rationale behind the structural design of these compounds. Among the synthesized derivatives, compounds bearing Schiff base functionality and electron-withdrawing aromatic substituents exhibited superior anticancer activity and selectivity, suggesting their potential as lead candidates. The results of molecular docking studies correlated well with in vitro cytotoxicity data, providing insights into the possible mechanism of action and target interactions. The study highlights the importance of rational structural modification of the thiazole scaffold to enhance anticancer potency while maintaining selectivity toward cancer cells. Overall, this research contributes valuable information to the field of medicinal chemistry and supports the continued exploration of thiazole derivatives as potential anticancer agents. Further studies involving in-depth mechanistic investigations, in vivo evaluation, and pharmacokinetic profiling are warranted to advance the most promising compounds toward preclinical and clinical development.

AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Conflict of Interests

The authors declare no conflict of interest

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Data Availability

Data will be available on request

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